User Manual :

# Gegenees V 1.0.1


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## What is Gegenees?

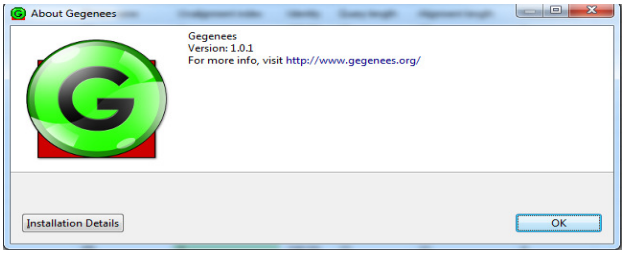
Gegenees is a software that compares genome sequences (Draft and Completed). It was primarily developed for bacterial genomes but it is also possible to use on viruses and smaller eukaryotes. Gegenees fragments the genomes and compares all pieces against all genomes. Based on this all-against-all comparison, a phylogenetic data can be extracted. It is also possible to define a "target group" and search for genomic regions that have high specificity for the target group. This is referred to as a "genomic signature". The genomic signature regions can be used to find candidate regions for the design of primers and probes for ne highly specific diagnostic assays. There is also a built in primer/probe verification system that compares new candidate or existing primers and probes to the

genomic database and to the defined target groups. Future versions will include more aspects of comparing next generation sequencing (NGS) data .

## Version system:

The first released version was 1.0.1. Based on user feedback, new versions will be released that solves problems and makes the program easier to use. These versions will be called 1.0.1, 1.0.2 .... New functionality will lead to version 1.1.1, 1.2.1 ....

To see your version, select "About Gegenees" in the Help main menu.




## What's new

This is the first official user manual.

## Installation:

The software can be downloaded from http://www.gegenees.org. There are several variants uploaded, depending on your operating system (OS). Windows. Macintosh and linux are supported. You must also chose the correct processor architecture (32 bit or 64 bit).

To see the architecture :

In Windows 7 , select "properties" when right clicking "computer".
In Macintosh, select " About this Mac" from the Apple menu. Mac OS X 10.5 (or greater) is a 64-bit.
In Linux, in a terminal, type "uname -a". If "x86_64" or "ia64" is shown, the system is 64 bit.

Java needs to be installed . You may check your Java version at this link: http://java.com/en/download/installed.jsp.

Download and extract the compressed Gegenees program. Run the Gegenees program from in the "Gegenes" folder. You will then be asked to specify a "Workspace". This is the place where all your genomes and comparisons will be stored. In the current version the workspace directory name SHOULD NOT CONTAIN SPACES. Neither should any of the directories "above" contain spaces. This is because the BLAST program uses spaces to separate command arguments. A version that is compatible with spaces is under development, but at this stage spaces should be avoided.

If Gegenees starts OK, it is time to install BLAST. BLAST can be downloaded from ftp://ftp.ncbi.nih.gov/blast/executables

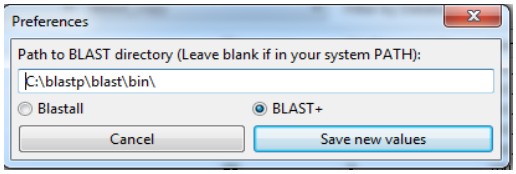
Gegenees can use both BLAST and BLAST+. In general, The latest version of BLAST+ is recommended.

ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/LATEST

Chose a version that corresponds to your OS and architecture (32 bit/64 bit) and extract (or in windows run the installation program). In the current version, the directory blast is installed should NOT CONTAIN SPACES. This requirement will be removed in future versions.

Gegenees must then be configured to find BLAST. Chose "preferences" from Gegenees main menu. Select blastall for older version of NCBI blast, 2.2.20 and older, and BLAST+ for newer (2.2.21 and later). If BLAST is added to your system path, you may not need to specify the path. If BLAST is not in the system path or if BLAST cannot be found by gegenees, specify the full pathname to the directory containing the BLAST executables.

e.g. Windows, C:\blast\ Linux, \usr\local \blast\




A function for testing if Gegenees finds blast is under implementation and will come in the next version.

## Perspectives

Gegenees have five "perspectives". The active perspective can be changed in the "perspective bar" just under the main menu. The perspectives are:

**Workbench overview :** An overview of all comparisons collected in this "workspace" (se below).

**Genomes:** An overview of the genomes available in the local database. Remote genomes at the NCBI ftp server can also be explored and downloaded. Genomes can also be copied into a the current comparison.

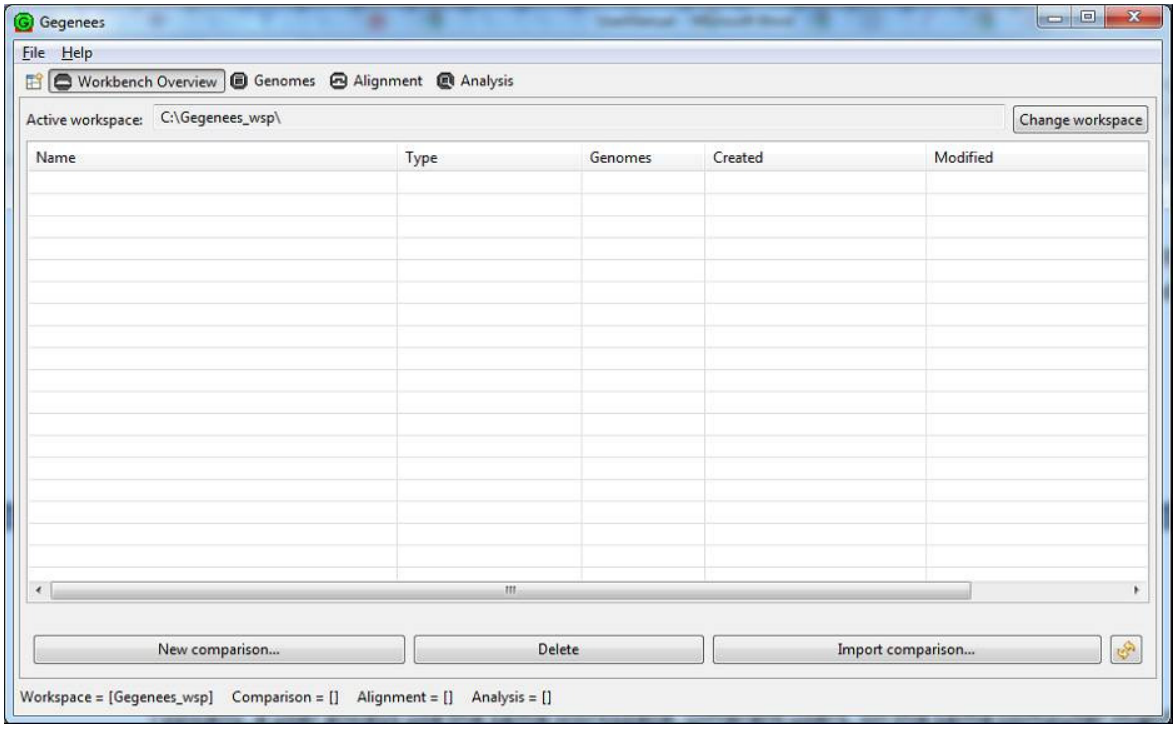
**Alignment:** This is the perspective where the comparative calculations are started and controlled.

**Analysis:** a perspective for phylogenomic and signature analysis of a completed alignment.

## The workspace

The workspace is where all your genomes and your comparison projects will be stored. A wokspace must always be selected, so when Gegenees starts, the first time a workspace location is asked for.

Typically, a user always use the same workspace. Different users, on the same computer might want to collect their genomes and comparison in an own, separate workspace. The workspace can be changed by pressing the "Change workspace" button. Do not use space characters in the workspace path. The full path of the workspace can be seen in the textbox "Active workspace". The name of the current workspace can always be seen at the bottom left side of the "status line". The comparisons that are present in the current workspace will be listed in the table in the Workbench overview perspective.




## The local database

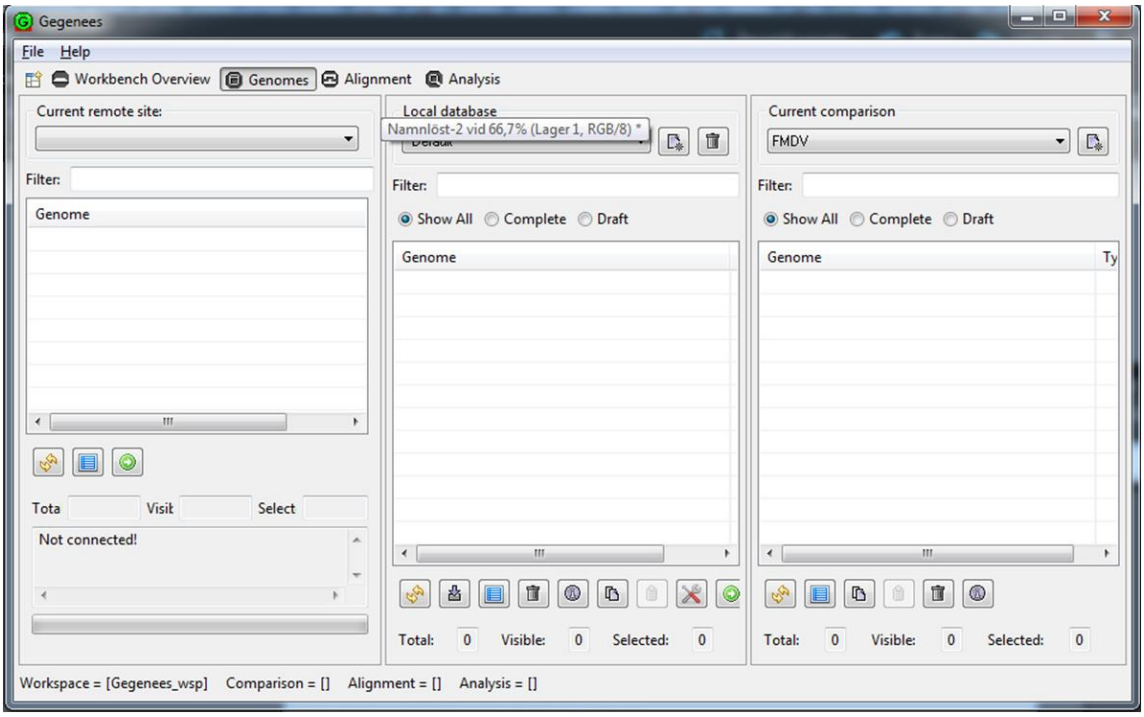
This is where you store your genomes. A "default" database is always present but customized databases can also be made. The default database represents a directory called "database" in the workspace. Custom databases represent directories named "database_NameOfCustomDatabase".

The local database content is shown in the middle part of the "Genomes perspective". If the database is large, subsets can be shown by entering a case sensitive filter text. (e.g Bacillus to show only the genus Bacillus). There is also a filter for showing only sequences in draft or complete form.

Below is a toolbar with functions for:

- refreshing database content from disk (if you make a manual import of genomes)
- Starting an import Wizard.
- Selecting all visible genomes (with the current filter)
- Deleting selected genomes.
- Bring up an information box about the selected genomes (size, GC content, nr contigs etc).

- Copy genomes, i.e. put genomes in an (Gegenees specific) clipboard.
- Paste genomes from the (Gegenees specific) clipboard.
- Change name or type (complete/Draft) for a genome
- copy to the current comparison (genomes can also be copied from a comparison into the local database if needed, e.g. if you get a ready-made comparison)




## Remote Sites

It is possible to download genomes from a remote FTP server. The FTP-sites are definend by a file ending with ".site" in the "ftp" directory in the workspace directory. A few pre-defined ftp sites are distributed with the Gegenees software. These are NCBI complete genomes (pointing at ftp://ftp.ncbi.nih.gov/genomes/Bacteria/) and NCBI genomes bacteria draft (pointing at ftp://ftp.ncbi.nih.gov/genomes/Bacteria_DRAFT/) and NCBI genbank bacteria draft (pointing at ftp://ftp.ncbi.nih.gov/genbank/wgs/) "NCBI genbank bacteria draft" contains more genomes than "NCBI genomes bacteria draft". It is possible to make own ".site" files. Copy the content of the existing files and replace the "HostName:" and the "Directory:" fields with custom information. The FTP site must be formatted as the NCBI site. If you need to connect to other types of ftp sites,contact the support at Gegenees.

## Gegenees genome format

Gegenees uses a folder for each genome. The folder name corresponds to the Name of the genome as appearing in Gegenees and the type of the genome ("Draft"/"Complete". The last part of the folder name has the format "--Draft--" or "--Complete". In future versions, more types may be introduced. The folder contains at least one Genbank formatted file with extension ".gbk" or ".gbff". If there are several subsequences/contigs, they may be in the same file or in separate files. Genomes are stored in the database folder(s) but also in the comparison folders. Thus. the comparison keeps a copy of the part of the database it is using. If the database is modified, the genomes belonging to a comparison is still untouched in the comparison folder.

## Gegenees comparison

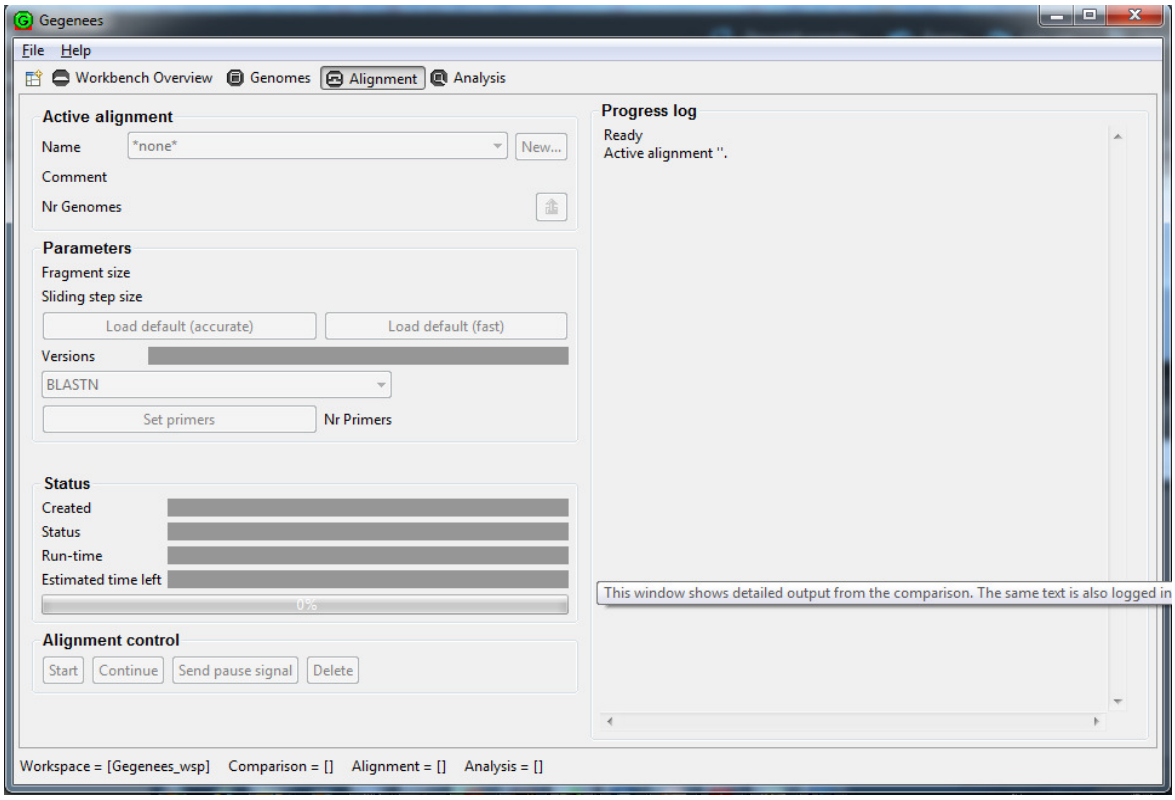
The Gegenees "comparison" is a "package" containing:

- a list of genomes that belongs to the comparison
- a copy of all the genomes as they looked like when they were added to the comparison
- typically, at least one "alignment" which represents a comparative calculation at a certain resolution (se below).
- Files coupled to the analysis of the alignment (s).

Thus, the first step when making an comparative analysis is to define the genomes. This can be done by copying the from the "local database" part in the "Genomes" perspective into the "current comparison" in the right part of the "Genomes" perspective. This is typically done with the "green arrow". The list of genomes and the genome copies are then produced. No alignment is created at this time. A comparison is represented by a folder with the prefix "comparison_" and it contains copies of the genomes and a file "comparison.geg" that contains the genome list.

## Gegenees alignment

When a comparison is defined, one or several alignments can be made. The alignment represents a comparative calculation thatn be made at different resolutions. An alignment is created in the "Alignment" perspective with the "New..." button. Two type of alignments can be made, "fragmented alignment" and "primer alignment". the primer alignment is a special case and is described below in a separate heading. The resolution of the alignment is controlled by two parameters, the "fragment size" and the "sliding step size". The fragment size represents the "scanning window size" and it should be smaller than the genomic region you anticipate to find in the analysis. For bacteria, we recommend 200 frag-size/100 slide-size (more accurate) and 500/500 (much faster and usually sufficient)settings. Small fragment sizes and sliding step sizes gives more demanding calculations. When working with viruses and small sequences, shorter settings may be needed. It is also possible to use tblastx (compares sequences on translated level, i.e aminocids). This is very much more demanding and the datasets should be smaller. If the sequences are pylogenetically far apart, this may be a useful operating mode. An alignment is represented on the disc by a folder with the prefix "alignment_".




When an alignment is defined it can be started by pressing the "start" button. The calculation progress will be shown and the log-window in the right part will show messages on what is happening. Typically, first a lot of conversion and preparation messages appears. Then the a BLAST list is created and executed in parallel "threads". Typically, each "thread" should not take more than at the most a few minutes to complete. The number of simultaneously calculating threads is indicated and also the thread number and the total number of threads that should be run. It is possible to send a pause signal and then resume the calculation later. After all the threads have been run, some data analysis is made and then the alignment is completed. Once a alignment is completed, it is possible to analyze the data in the "Analysis" perspective. There is an export button after the "Nr of genomes" field that can be used to export a list of accession numbers for the genomes.

## Primer alignment

Primer alignments is a special type of alignment where primers and/or probes are used instead of fragments. This is usually not as demanding calculation as fragmented alignments. The primer alignment is analyzed in a special tab in the analysis perspective (see below).

## Analysis

The analysis perspective contains different tools for analyzing the data from a completed alignment. The tabs are:

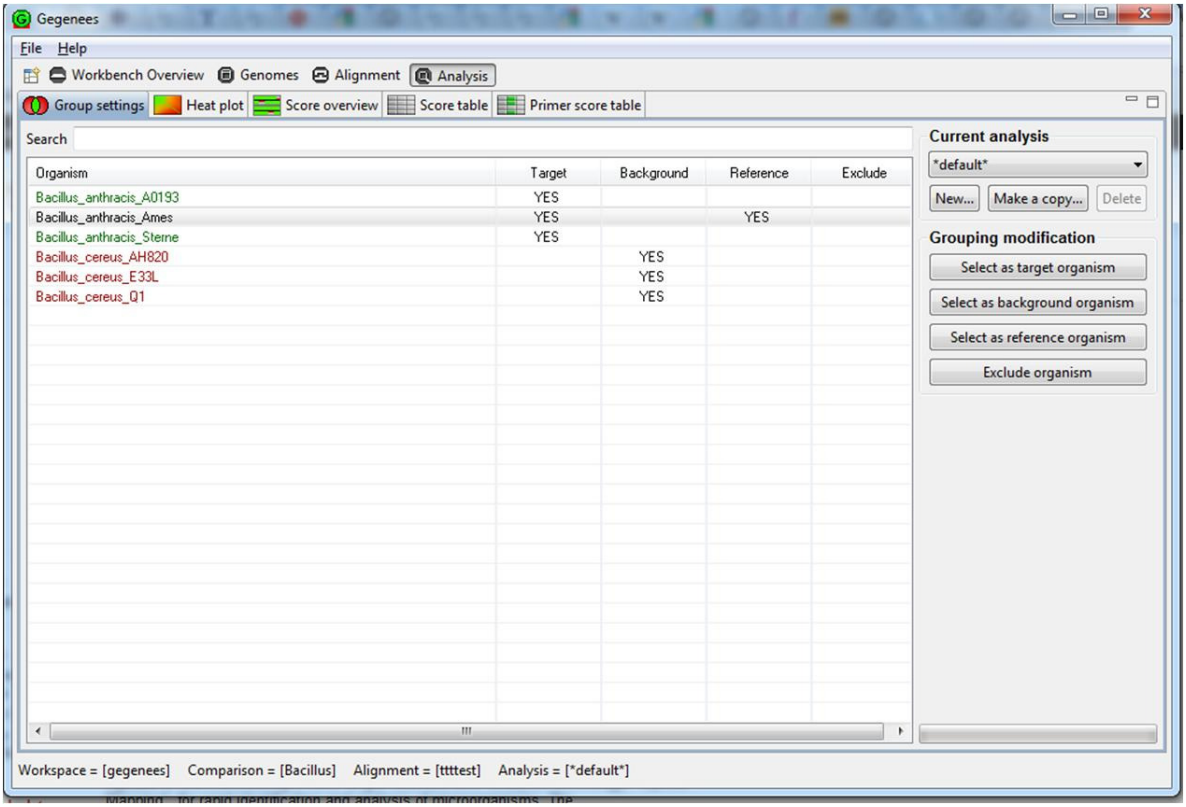
| | |
|---|---|
| Group settings: | A tabular view of the genomes that allows definition of the target and background groups for the analysis. |
| Heat plot: | This tab represents an phylogenomic overview of the data based on average similarities. |
| Score overview: | This tab represents a graphical overview and exploration tool of the genomic regions that are unique or discriminatory for the target group. |
| Score table: | This tab represents a tabular exploration tool for the genomic regions that are unique or discriminatory for the target group. |
| Primer score table: | This tab allows analysis of "primer alignments" to compare specificity of primers and probes based on the genomic data and the target group settings. |

## Group settings tab

The group settings tab allows the target group and the background group to be define.

- The target group represents the genomes that you are interested in. You might want to find discriminatory genomic regions that can be used to design specific molecular assays. You might also be interested in finding and analyzing genomic regions that are associated with a specific phenotypic trait that the target group share. The target group should ideally contain genomes that represents the genomic variability in the group as well as possible.
- The background group represents other genomes. The background should contain the genomes that are most likely to give a cross-reaction (false positive in an assay). For best result, close neighbor strains/isolates that do not contain the phenotypic trait should be included.
- Excluded genomes. You may also chose to exclude genomes from the analysis.
- The reference genome is the genome where the nucleotide coordinate system and the annotations are collected from. For a high stringency biomarker analysis (se below), the best annotated genome in the target group should be used. For lower stringencies in the biomarker analysis, the result may be slightly different depending on which target genome is selected as reference. It is then good to look at the data with different reference genomes. **A reference genome must be chosen to be able to look at the score overview and score table tabs.**

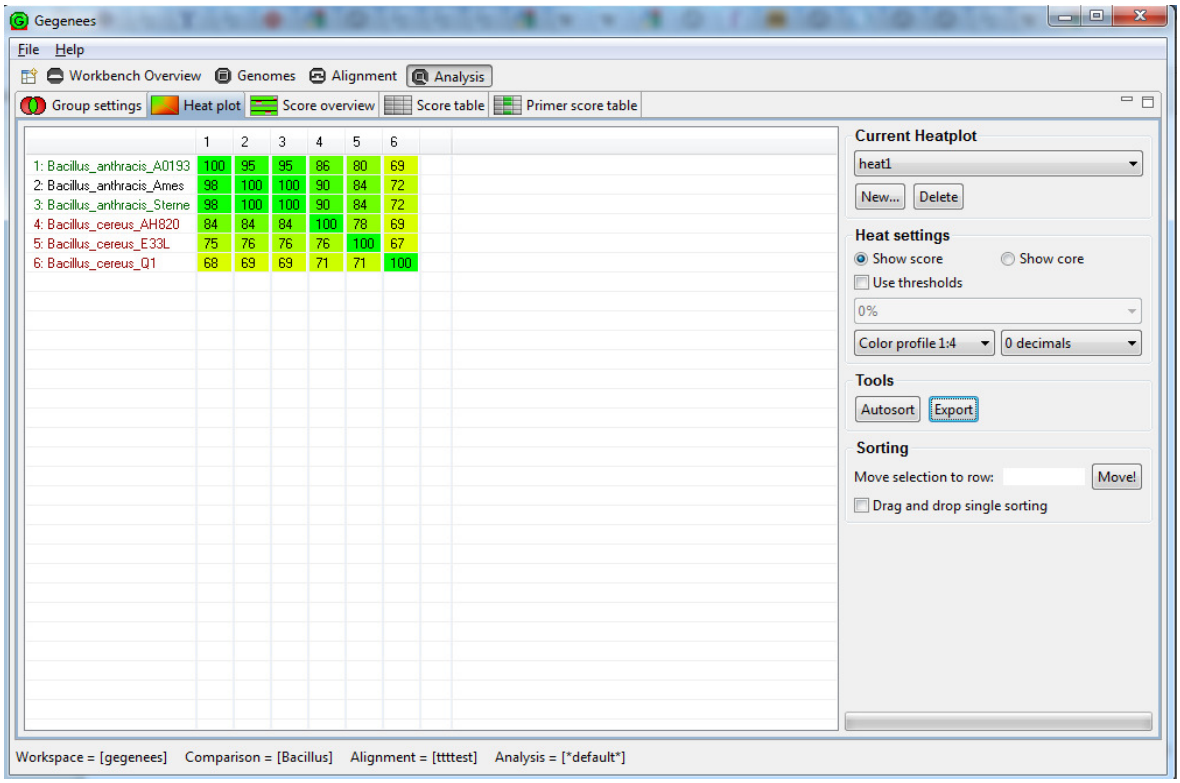
Several group settings can be created from the same dataset (e.g. different subtypes) with the "New.." or "Make a copy..." buttons.




## Heat plot tab

The heat  plot tab gives an phylogenomic overview of the data.  shown. The average normalized BLAST score values of all fragments are shown. It is also possible to define threshold values, meaning that that fragments falling under the threshold is not used to calculate the average similarity value. This gives a better phylogenetic  signal since the similarity value is only based on conserved genetic material (the core genome). It is also possible to see how large the core genome is at the specified threshold (select "show core" insted of "show score"). It is possible to change the "color profile" of the heatplot so that differences are highlighted as well as possible for the particular dataset. The number of decimals shown can be changed. The genomes are sorted alphabetically, which often is sufficient. There are sorting possibilities built in for the heat plots. It is possible to move genomes or group of genomes with the "Move selection to row" field or by right clicking it and select "move" from the context menu. The target and background group settings can also be modified from the right click-context menu.  If, "Drag and drop, single sorting" is selected, genomes can be draged with the mouse, one by one. The sort is saved and if several sorts are wanted, new ones can be created with the "new..." button. There is also an "autosort" function, that tries to minimize the distances between the rows. There is also an export button that allows of the phylogenomic data:

- export of the table in tab-format (for work in spreadsheet programs)
- export as html. Can be opened in a web browser or converted to publication grade figures. This is sometimes a better overview if the table is very large.
- export nexus file. Use this export to create dendrograms in e.g. SplitsTree .




## Score overview tab

The score overview tab shows a graphic representation of the "biomarker scores". biomarker scores are score values that rank all genomic regions (fragments) in how discriminating they are for the target group in terms of conservation (no false negatives) and uniqueness (no false positives in the background). There are three types of scores with different stringencies:

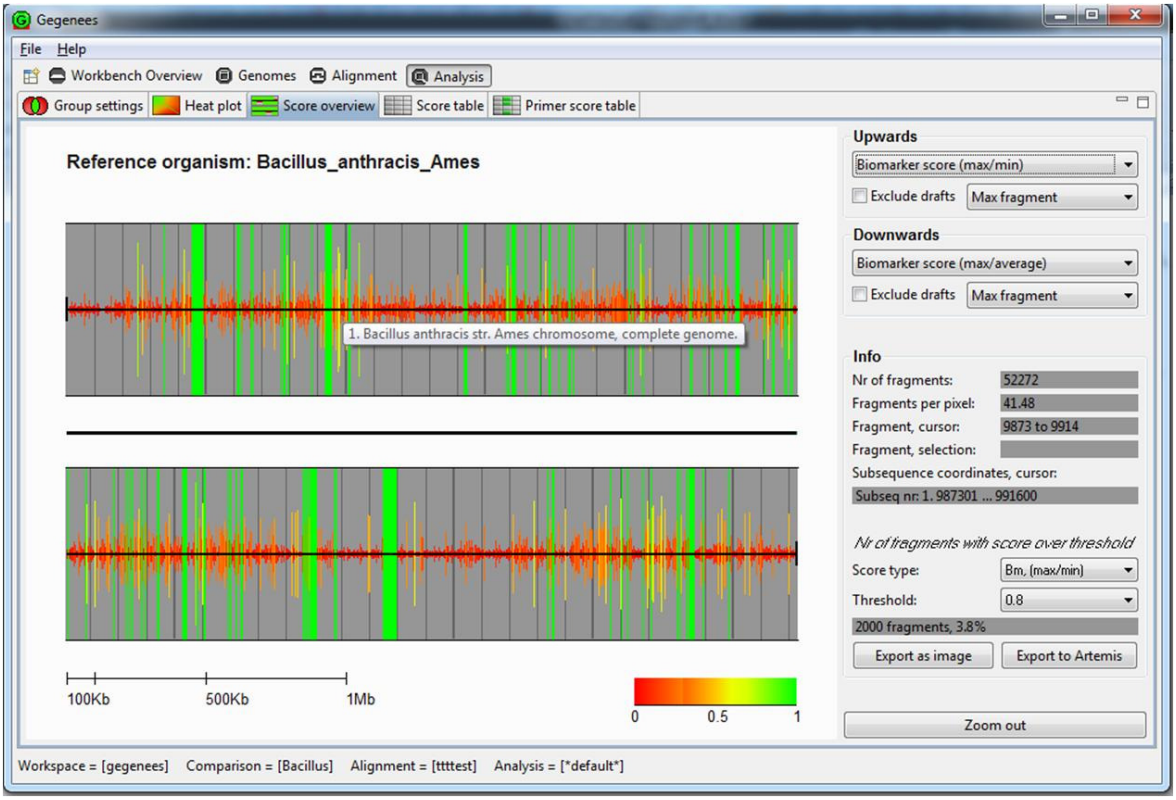
| | |
|---|---|
| Biomarker score (max/min): | represents the highest stringency. The high scoring fragments must be present and conserved in ALL target group genomes and must be absent or very diverged in ALL background genomes. The score goes from 0 (or negative values) representing bad regions up to 1 which represents a perfect region. The score is based on the worst background genome (max score) and on the worst target group genome (min score). |
| Biomarker score (max/average) | Similar to Biomarker score (max/average) but uses average values of the background group (relaxes the uniqueness criterion) |
| Biomarker score (average/average) | Similar to Biomarker score (max/average) but uses average values of the background group and the target group (relaxes the uniqueness and the conservation criterion) |

| | |
|---|---|
| Target group maximum score | Shows the best score in the target group (self score). This is usually 100% everywhere, but if bad regions are present in the sequence (e.g. nnnnnnnnnnnnn), they can be identified here. |
| Target group minimum score | Shows the worst conservation within the target group. Can be used to find highly conserved regions. |
| Target group average score | Shows the average conservation within the target group. Can be used to find highly conserved regions. |
| Background group maximum score | Shows the best score in the background group. This represent the worst cross reaction. |
| Background group minimum score | Shows the minimum score in the background group. Can be used to find regions that are not conserved in a group of very related genomes. |
| Background group average score | Shows the average conservation within the background group. |

The biomarker scores are drawn graphically. There is possibilities to compare two types of scores by drawing one upwards (from the coordinate axis) and a second downwards. The graphical view is spitted into two rows in order to use the computer screen optimal (do not confuse the upper row with the upwards score, there are upwards scores in both rows) . there is a possibility to exclude draft genomes in the calculations since they sometimes lack regions that in some cases may disturb the analysis. When the mouse moves over the graph, the subsequence,fragment number and coordinate at the cursor position is shown in the "info" part to the right. The number of fragments that each pixel column on the screen represent, is also indicated. It is also possible to see how many percent of the genome has a biomarker score over a certain threshold. This gives a good overview of the how much genomic regions one can expect.

It is possible to zoom into the graph by selecting a region with the right mouse button down. If the left mouse button is used, the corresponding region is selected. A selection can thereafter be loaded into the tabular view for further data mining.

There are  possibilities to export the graph (as seen on the screen) as an image. It is also possible to export the data to a file that can be explored in Artemis (se section below).




## Viewing a signature in Artemis

It is possible to export an interesting subsequence from the genome (or the whole genome if it is completed) into a format that can be viewed in Artemis http://www.sanger.ac.uk/resources/software/artemis/.

The export will end up in a directory called "export" under the workspace directory. It will be a "*.gbk" file that essentially is the same file as the original "gbk" file (if there are problems or warnings when loading the original file in Artemis they will remain). The "gene" and "misc feature" track is replaced by the biomarker scores. Five files are exported

1. the original annotated file with also the gene and misc feature track intact.
2. a file with Biomarker scores (max/min) as a misc_feature track
3. a file with Biomarker scores (max/avr) as a misc_feature track
4. a file with Bimarker scores (avr/avr) as a misc_feature track
5. a file with Biomarker scores (max/min), only complete genomes as a misc_feature track

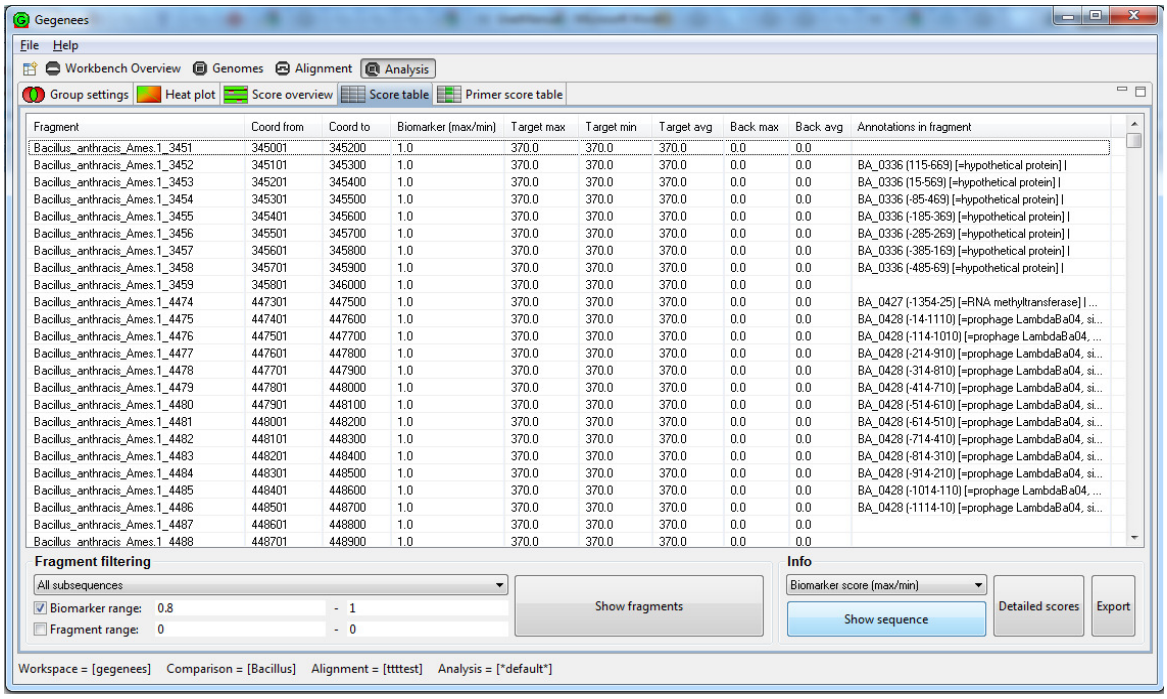
an example of how this kind of export will look like in artemis is shown below.

## Score table tab

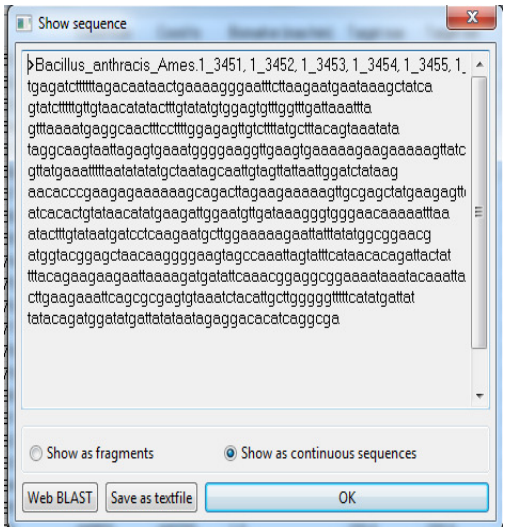
In the "score table" tab, details about the fragments representing either

- a selection (e.g. a range defined by the mouse in the graphical view)
- a user defined range (expressed as fragment numbers)
- a subset of fragments with a specific biomarker score-range (e.g. 0.8-1.0)
  (1.0 is the maximum biomarcer score)
- If none of the range boxes are checked, all fragments will be shown

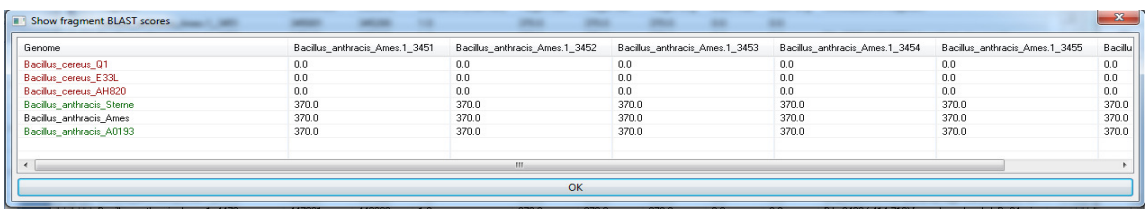
Fragments from a certain subsequence can also be selected. After setting the filtering range (or a combination of them, e.g. all fragments in the first 200 kb of subsequence 2 with biomarker sore over 0.8)press the "show fragments" button to load the fragments. It is possible to sort the table based by clicking the header. The type of biomarker score to be shown can be selected in the info region.




Show sequence displays the actual sequences of the fragments and it is possible to fuse adjacent and overlapping fragments into continuous sequences. The sequences can be exported to a Fasta-file or sent to a web page ready for a blast comparison at NCBI.



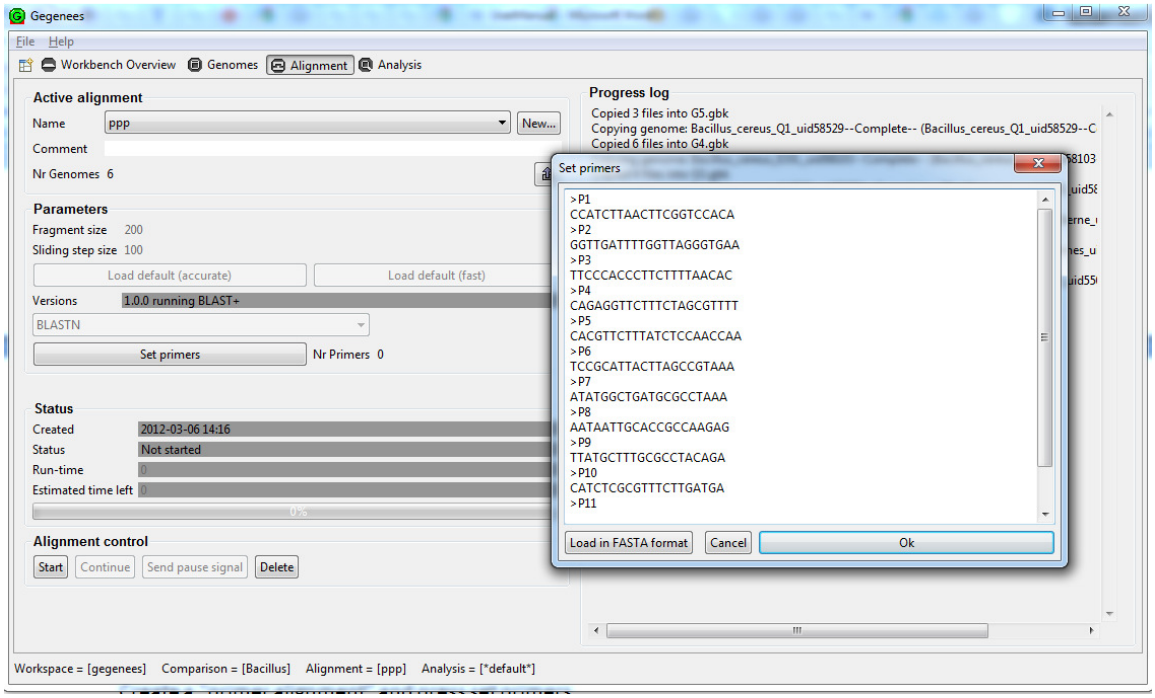

The detailed scores, shows how each fragment scores against each genome in the target and background group. This may help to identify which particular strain is causing a cross reaction.



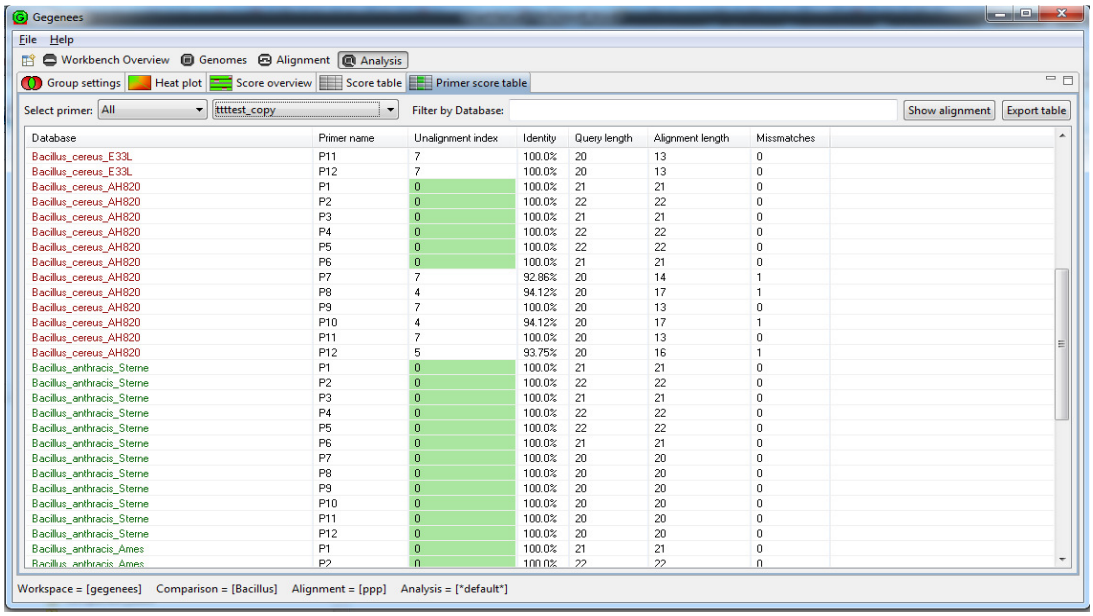
It is also possible to export the table (as its shown) or the full data table (without filtering) as a tab delimited text file for further analysis in e.g. a spreadsheet program.

## Primer alignment/Primer score table tab

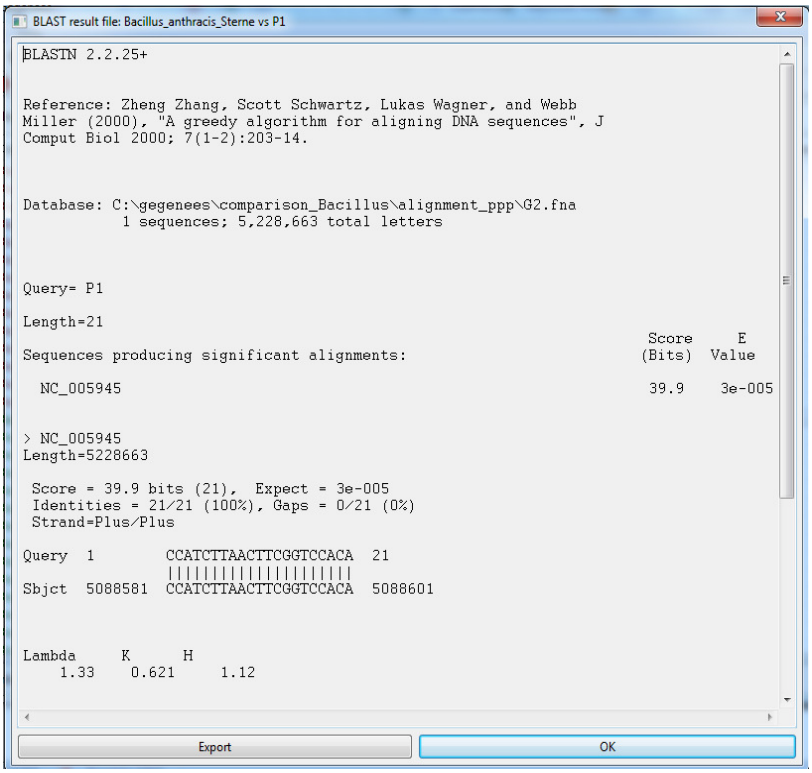
To make a primer/probe analysis, create or use an existing comparison with the genomes of interest. Create a "primer alignment" and press "set primers". enter your primers and run the analysis.




The primer alignment can now be explored in the "Primer score table tab". The "unalignment" index, represents missmatches in the alignment plus non-aligned nucleotides. It is marked green if it is a perfect match. A target group/ background group setting can be loaded from a fragmented alignment in the same comparison, and the genomes are color coded accordingly, so that a the primer matching can easily be compared to the target group definition.


Gegenees
File Help
Workbench Overview Genomes Alignment Analysis
Group settings Heat plot Score overview Score table Primer score table
Select primer: All ttttest_copy Filter by Database: Show alignment Export table
Database Primer name Unalignment index Identity Query length Alignment length Missmatches
Bacillus_cereus_E33L P11 7 100.0% 20 13 0
Bacillus_cereus_E33L P12 7 100.0% 20 13 0
Bacillus_cereus_AH820 P1 0 100.0% 21 21 0
Bacillus_cereus_AH820 P2 0 100.0% 22 22 0
Bacillus_cereus_AH820 P3 0 100.0% 21 21 0
Bacillus_cereus_AH820 P4 0 100.0% 22 22 0
Bacillus_cereus_AH820 P5 0 100.0% 22 22 0
Bacillus_cereus_AH820 P6 0 100.0% 21 21 0
Bacillus_cereus_AH820 P7 7 92.86% 20 14 1
Bacillus_cereus_AH820 P8 4 94.12% 20 17 1
Bacillus_cereus_AH820 P9 7 100.0% 20 13 0
Bacillus_cereus_AH820 P10 4 94.12% 20 17 1
Bacillus_cereus_AH820 P11 7 100.0% 20 13 0
Bacillus_cereus_AH820 P12 5 93.75% 20 16 1
Bacillus_anthracis_Sterne P1 0 100.0% 21 21 0
Bacillus_anthracis_Sterne P2 0 100.0% 22 22 0
Bacillus_anthracis_Sterne P3 0 100.0% 21 21 0
Bacillus_anthracis_Sterne P4 0 100.0% 22 22 0
Bacillus_anthracis_Sterne P5 0 100.0% 22 22 0
Bacillus_anthracis_Sterne P6 0 100.0% 21 21 0
Bacillus_anthracis_Sterne P7 0 100.0% 20 20 0
Bacillus_anthracis_Sterne P8 0 100.0% 20 20 0
Bacillus_anthracis_Sterne P9 0 100.0% 20 20 0
Bacillus_anthracis_Sterne P10 0 100.0% 20 20 0
Bacillus_anthracis_Sterne P11 0 100.0% 20 20 0
Bacillus_anthracis_Sterne P12 0 100.0% 20 20 0
Bacillus_anthracis_Ames P1 0 100.0% 21 21 0
Bacillus_anthracis_Ames P2 0 100.0% 22 22 0
Workspace = [gegenees] Comparison = [Bacillus] Alignment = [ppp] Analysis = [*default*]

If a primer row is double-clicked with the mouse or the "show alignment" button is pressed, the alignment of this primer against this genome is shown. The table and the alignment views can also be exported as text files.


BLAST result file: Bacillus_anthracis_Sterne vs P1
BLASTN 2.2.25+
Reference: Zheng Zhang, Scott Schwartz, Lukas Wagner, and Webb Miller (2000), "A greedy algorithm for aligning DNA sequences", J Comput Biol 2000; 7(1-2):203-14.
Database: C:\gegenees\comparison_Bacillus\alignment_ppp\G2.fna
1 sequences; 5,228,663 total letters
Query= P1
Length=21
Sequences producing significant alignments: Score (Bits) E Value
NC_005945 39.9 3e-005
> NC_005945
Length=5228663
Score = 39.9 bits (21), Expect = 3e-005
Identities = 21/21 (100%), Gaps = 0/21 (0%)
Strand=Plus/Plus
Query 1 CCATCTTAACTTCGGTCCACA 21
Sbjct 5088581 CCATCTTAACTTCGGTCCACA 5088601
Lambda K H
1.33 0.621 1.12
Export
OK